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Within-Person Variability of Urinary Bisphenol-A in Postmenopausal Women

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Abstract

We evaluated the within-person variability of urinary BPA levels over two samples collected three years apart in 90 Women's Health Initiative participants. The intraclass correlation coefficient was 0.09 (95% CI 0.01–0.44), indicating high within-participant variability relative to the between-person variation. Concordance of BPA quartile over time was low (31.7%) and was unrelated to demographic, behavioral, or dietary factors. A single, or even several, measurements of BPA may not adequately classify long-term exposure in human studies.

Keywords

Bisphenol-A; variability; exposure measurement; women; postmenopausal

Introduction

Nearly all U.S. residents have measurable urinary BPA, though levels range widely (Calafat et al. 2008). Exposure occurs primarily through leaching of BPA from plastic containers and cans into foods and liquids that are subsequently ingested (Vandenberg et al. 2007).

BPA's role as an endocrine disruptor has raised concerns that exposure to BPA may increase risk of various health outcomes, including infertility, diabetes, and obesity (Rochester 2013). However, human studies of BPA's effects are lacking, perhaps due to the high within-person variability of BPA (Mahalingaiah et al. 2008; Nepomnaschy et al. 2009; Townsend et al.

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2013; Ye et al. 2011). BPA is metabolized quickly, with 50% excreted in the urine after 6 hours (Volkel et al. 2002). A single measurement of BPA may not accurately classify an individual's usual exposure, and it is unknown if incorporating BPA levels from multiple timepoints will improve classification. Also, whether certain subgroups (e.g. individuals of a healthy weight) might have more stable levels over time is unclear. If so, such information could help refine BPA exposure assessments by identifying populations most or least likely to have stable BPA levels. Improved exposure assessment is critical for studies seeking to relate BPA exposure to human health outcomes. We addressed these important questions in a sample of postmenopausal women participating in the Women's Health Initiative (WHI).

Methods

The WHI has been described previously (1998). Briefly, the WHI recruited postmenopausal women nationwide into Clinical Trial (CT; N=68,132) or Observational Study (OS; N=93,676) groups. Participants were 50 to 79 years at enrollment and provided data through annual in-person clinic visits (CT) or through annual mailed questionnaires and in-person clinic visits every three years (OS). Questionnaire data included information on demographic characteristics, medical history, and health behaviors in addition to other characteristics. All participants provided written informed consent, and the WHI was approved by Institutional Review Boards at each clinical center and conducted following the Declaration of Helsinki.

Three WHI sites (Birmingham, AL; Pittsburgh, PA; Tucson, AZ) participated in a bone density substudy in which all participants provided first morning void urine samples at each clinic visit; we randomly selected 15 OS women and 15 CT women with available urine from each of these three sites (N=90). Eligible women had no incident cancer, stroke, or coronary heart disease through year 3 of their WHI follow-up. OS participants provided two samples (baseline, year 3) and CT participants provided three samples (baseline, year 1, year 3) for analysis.

Total and unconjugated urinary BPA were measured by the Analytical Biochemistry Shared Resource/University of Hawaii Cancer Center (AAF) by isotope dilution (BPA- $^{13}\text{C}_{12}$ obtained from Cambridge Isotope Laboratory, Andover, MA) high performance liquid chromatography orbitrap mass spectrometry (HPLC/MS). Unconjugated and total BPA were determined by direct extraction from urine and by enzymatic hydrolysis (glucuronidase/sulfatase treatment) followed by urinary extraction, respectively. Extracts were dansylated prior to LC/MS injection to improve sensitivity. Calibration was linear to 50 ng/mL with a detection limit of 0.02 ng/mL. Because unconjugated BPA levels were very low (median 0.5 ng/mg creatinine), suggesting low probability of post-void contamination (Ye et al. 2007), we used total BPA levels for our analyses. Creatinine was measured using a Roche-Cobas MiraPlus clinical auto-analyzer and a kit from Randox Laboratories (Crumlin, UK). Coefficients of variation (CV) of 17% and 7.3% were obtained for total BPA and creatinine, respectively.

BPA levels were divided by creatinine values to correct for urine dilution. We calculated the median and range for total BPA at each time overall and by WHI cohort. Differences

between cohorts and across time were tested using a Wilcoxon signed rank test. We generated scatter plots of total BPA measurements by repeated timepoints. The intra-class correlation coefficient (ICC) was calculated overall and by WHI cohort using a linear mixed effects regression model in SAS. Power calculations for the detectable ICC were made using the appropriate routine in PASS (Hintze 2006). Quartiles of total BPA were defined using the baseline values in the full population. Year 1 and year 3 measurements also were categorized using the baseline quartile ranges. Participants were classified as “concordant” if their total BPA levels were in the same quartile upon repeated measurement, or as “discordant” otherwise. Baseline demographic and health behavior characteristics (total BPA level, age, race, education, hormone therapy use, body mass index, smoking status, alcohol use, physical activity, daily servings of grains, fruits, vegetables, dairy, meats, beans, tomatoes, soup, and canned tuna, and a Healthy Eating Index (HEI) score (U.S. Department of Agriculture)), were compared between concordant and discordant groups using t tests and chi square tests. We repeated analyses defining “adjacent concordance” as changing to an adjoining quartile. Analyses were conducted using SAS version 9.0 (SAS, Cary, NC) and Stata version 13.0 (Stata Corporation, College Station, TX).

Results

After excluding 1 outlier (total BPA >100 ng/mg creatinine), 5 samples where measured free BPA was implausibly greater than total BPA, and 3 samples in which creatinine could not be assessed due to insufficient sample volume, total urinary BPA levels were available for 86 baseline samples, 44 year 1 samples (CT only), and 86 year 3 samples. Total BPA levels were similar between CT and OS cohorts (data not shown). Median total BPA levels were similar over time (Table 1), though levels varied considerably within-participants over a three-year period (Figure 1). The ICC was 0.09 (95% CI 0.01–0.44) in the total population, 0.06 (95% CI 0.00–0.43) in the OS, and 0.08 (95% CI 0.01–0.53) in the CT. Similar results were obtained when ICCs were calculated using raw total BPA values with adjustment for creatinine levels (data not shown).

Quartile ranges defined by total urinary BPA levels (ng/mg creatinine) at baseline were: Q1, 2.41; Q2, 2.42– 3.25; Q3, 3.26– 5.36; and Q4, >5.36. Concordance from baseline to year 3 was 37.1%. Among CT participants, concordance was 42.9% from baseline to year 1 and 23.3% from years 1 to 3.

Only having an annual household income greater than \$50,000 was predictive of concordance over a three-year period, though this result was of borderline significance ($p=0.06$) (Table 2). Additionally, consumption of specific food groups or items was unrelated to concordance (data not shown). Similarly, these characteristics were not predictive of concordance over one or two years in the CT, except for an association between moderate exercise and one-year concordance ($p=0.02$; data not shown). Sixty-seven percent of participants were considered “adjacent concordant,” and this also was unrelated to any demographic or behavioral characteristics evaluated (data not shown).

Discussion

In this population-based sample of postmenopausal women, we observed high variability in urinary BPA levels over a three-year period. This result is consistent with previous studies in other populations that have reported high within-person variability of urinary BPA levels (Nepomnaschy et al. 2009; Townsend et al. 2013; Ye et al. 2011). We observed only slight improvement in the ICC with three versus two measurements over the same time period. Together with prior work, these results demonstrate that a single urinary BPA measurement, or even multiple measurements years apart, is likely insufficient for capturing true, usual BPA exposure in human studies.

Of particular note is that BPA levels three years apart were in a similar range in only 37.1% of participants, and concordance did not appreciably improve in samples separated by one (42.9%) or two years (23.3%). We found no clear evidence that any demographic characteristics or health behaviors were related to stable BPA levels over time. These results are consistent with BPA levels being most influenced by very recent exposures, such as diet. Our dietary measures captured “typical” diet, rather than foods consumed within the previous day, which likely is more related to BPA levels.

Indeed, exposure to BPA is primarily through diet, such as through leaching of BPA into canned foods (Noonan et al. 2011) and in some fresh fruits and vegetables (Vivacqua et al. 2003), though non-dietary sources of BPA are increasingly recognized (Vandenberg et al. 2007). Because BPA is rapidly metabolized and excreted by the body, measured urinary BPA levels represent very recent exposure; changes in dietary patterns and exposure to other sources likely contribute to the high within-person variability of urinary BPA measures. For example, Carwile *et al* observed that urinary BPA levels increased markedly with daily consumption of canned soup as compared to fresh soup (Carwile et al. 2011). The high within-person variability of urinary BPA would cause non-differential misclassification of exposure and attenuate results of epidemiologic studies, thus underestimating the true association between BPA exposure and various human health outcomes.

Strengths of our study include the use of an established, population-based cohort as opposed to highly selected populations, such as infertile adults (Mahalingaiah et al. 2008).

Additionally, the availability of extensive covariate data supported the evaluation of predictors of stability in urinary BPA levels over time. An important limitation is that only one of the three sites documented using BPA-free containers. However, our results showed minimal unconjugated BPA, suggesting that post-void contamination was not a significant contributor to the measured total BPA levels as conjugation only occurs from endogenous exposure (Ye et al. 2007). Our small sample size is also a limitation, however, we did have 80% power to detect an ICC of 0.40 (95% CI 0.19–0.58) as statistically significant. Finally, use of a first morning void sample may have failed to capture the peak of excreted BPA, as the primary source of BPA exposure is dietary, and approximately 50% of BPA is excreted within six hours (Volkel et al. 2002). Thus, BPA encountered through the diet may largely be excreted during the daytime and early evening hours, and would not be present in large quantities in a first morning void urine sample.

These results underscore the challenges of measuring BPA exposure in epidemiologic studies and caution that even multiple measurements spaced years apart may be insufficient to correctly classify exposure. Future work should focus on novel approaches to assessing BPA exposure, such as repeated daily measurements over a short time period or identification of genetic polymorphisms to distinguish fast-metabolizers from slow-metabolizers. At a minimum, until improved methods of exposure assessment are available, the high variability of urinary BPA levels should be considered when designing epidemiologic studies.

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Abbreviations

BPA	Bisphenol-A
CT	Clinical Trial
ER	Estrogen receptor
HEI	Healthy Eating Index
ICC	Intra-class correlation coefficient
OS	Observational Study
WHI	Women's Health Initiative

References

- Calafat AM, Ye X, Wong LY, Reidy JA, Needham LL. Exposure of the U.S. population to bisphenol A and 4-tertiary-octylphenol: 2003–2004. *Environ Health Perspect.* 2008; 116(1):39–44. [PubMed: 18197297]
- Carwile JL, Ye X, Zhou X, Calafat AM, Michels KB. Canned soup consumption and urinary bisphenol A: a randomized crossover trial. *Jama.* 2011; 306(20):2218–2220. [PubMed: 22110104]
- Hintze, J. NCSS, PASS, and GESS. Kaysville, Utah: NCSS; 2006.
- Mahalingaiah S, Meeker JD, Pearson KR, Calafat AM, Ye X, Petrozza J, et al. Temporal variability and predictors of urinary bisphenol A concentrations in men and women. *Environ Health Perspect.* 2008; 116(2):173–178. [PubMed: 18288314]
- Nepomnaschy PA, Baird DD, Weinberg CR, Hoppin JA, Longnecker MP, Wilcox AJ. Within-person variability in urinary bisphenol A concentrations: measurements from specimens after long-term frozen storage. *Environ Res.* 2009; 109(6):734–737. [PubMed: 19463991]
- Noonan GO, Ackerman LK, Begley TH. Concentration of bisphenol A in highly consumed canned foods on the U.S. market. *J Agric Food Chem.* 2011; 59(13):7178–7185. [PubMed: 21598963]
- Rochester JR. Bisphenol A and human health: a review of the literature. *Reproductive toxicology.* 2013; 42:132–155. [PubMed: 23994667]
- Townsend MK, Franke AA, Li X, Hu FB, Eliassen AH. Within-person reproducibility of urinary bisphenol A and phthalate metabolites over a 1 to 3 year period among women in the Nurses' Health Studies: a prospective cohort study. *Environ Health.* 2013; 12(1):80. [PubMed: 24034517]
- U.S. Department of Agriculture. [Accessed June 17, 2014] Healthy Eating Index - 2005. Available: www.cnpp.usda.gov/HEI/healthyeatingindex2005factsheet.pdf
- Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV. Human exposure to bisphenol A (BPA). *Reprod Toxicol.* 2007; 24(2):139–177. [PubMed: 17825522]
- Vivacqua A, Recchia AG, Fasanella G, Gabriele S, Carpino A, Rago V, et al. The food contaminants bisphenol A and 4-nonylphenol act as agonists for estrogen receptor alpha in MCF7 breast cancer cells. *Endocrine.* 2003; 22(3):275–284. [PubMed: 14709801]
- Volkel W, Colnot T, Csanady GA, Filser JG, Dekant W. Metabolism and kinetics of bisphenol a in humans at low doses following oral administration. *Chem Res Toxicol.* 2002; 15(10):1281–1287. [PubMed: 12387626]
- Women's Health Initiative Study Group. Design of the Women's Health Initiative clinical trial and observational study. *Control Clin Trials.* 1998; 19(1):61–109. [PubMed: 9492970]
- Ye X, Bishop AM, Reidy JA, Needham LL, Calafat AM. Temporal stability of the conjugated species of bisphenol A, parabens, and other environmental phenols in human urine. *J Expo Sci Environ Epidemiol.* 2007; 17(6):567–572. [PubMed: 17410114]
- Ye X, Wong LY, Bishop AM, Calafat AM. Variability of urinary concentrations of bisphenol A in spot samples, first morning voids, and 24-hour collections. *Environ Health Perspect.* 2011; 119(7):983–988. [PubMed: 21406337]

Figure 1.
Scatter plot of repeated measures of total urinary BPA at baseline and year 3.

Table 1

Median and range of total urinary BPA levels (ng/mg creatinine) by study arm and year of sample.

Timepoint	N	Median (5 th –95 th percentile)	P values ^a
Baseline	86	3.3 (1.5–7.5)	
Year 1 ^b	44	2.9 (1.3–11.9)	0.74
Year 3	86	3.4 (1.0–11.0)	0.57; 0.46

^aP values are comparison to Baseline; for Year 3, the second P value is for comparison to Year 1 among CT participants.

^bYear 1 measurements available in CT participants only.

Table 2

Baseline characteristics of study population by concordance of total urinary BPA quartile at Baseline and Year 3.

Characteristic ^a	Total Population N=82	Concordant N=26	Discordant N=56	P value ^b
Total urinary BPA, ng/mg creatinine; Mean (SD)	3.7 (1.8)	3.8 (1.8)	3.7 (1.8)	0.78
Age, years; Mean (SD)	63.4 (7.2)	63.2 (7.2)	63.5 (7.3)	0.84
50–59; N (%)	25 (30.5)	8 (30.8)	17 (30.4)	
60–69	39 (47.6)	12 (46.2)	27 (48.2)	
70–79	18 (22.0)	6 (23.1)	12 (21.4)	
White ethnicity; N (%)	64 (78.1)	22 (84.6)	42 (75.0)	0.33
Household income \$50,000; N (%)	17 (23.9)	9 (37.5)	8 (17.0)	0.06
Education; N (%)				0.64
High school or less	25 (30.9)	8 (30.8)	17 (30.9)	
Some college or college degree	30 (37.0)	8 (30.8)	22 (40.0)	
Post-graduate degree	26 (32.1)	10 (38.5)	16 (29.1)	
Hormone therapy use; N (%)				0.24
Never	26 (32.5)	7 (28.0)	19 (34.6)	
Past	23 (28.8)	5 (20.0)	18 (32.7)	
Current	31 (38.8)	13 (52.0)	18 (32.7)	
Body mass index, kg/m ² ; Mean (SD)	27.2 (5.7)	25.9 (4.8)	27.9 (6.1)	0.15
Normal/underweight (<25.0 kg/m ²); N (%)	33 (40.2)	13 (50.0)	20 (35.7)	0.47
Overweight (25.0 – <30.0 kg/m ²)	27 (32.9)	7 (26.9)	20 (35.7)	
Obese (≥ 30.0 kg/m ²)	22 (26.9)	6 (23.1)	16 (28.6)	
Past or current smokers; N (%)	35 (44.3)	12 (46.2)	23 (43.4)	0.82
Consume 1 alcoholic drink per week; N (%)	25 (30.5)	8 (30.8)	17 (30.4)	0.97
Walking, MET-hours per week; Mean (SD)	4.2 (5.1)	3.8 (4.0)	4.3 (5.6)	0.72
Mild exercise, MET-hours per week; Mean (SD)	1.9 (3.5)	2.1 (4.7)	1.7 (2.8)	0.62
Moderate exercise, MET-hours per week; Mean (SD)	2.5 (4.5)	2.9 (5.0)	2.4 (4.2)	0.61
Hard exercise, MET-hours per week; Mean (SD)	4.3 (9.4)	6.3 (11.0)	3.3 (8.4)	0.19
Healthy Eating Index; Mean (SD)	67.2 (10.7)	67.2 (10.0)	67.2 (11.1)	0.98

^aMissing data are as follows: income, n=11; education, n=1; smoking status, n=3; physical activity variables, n=1

^bP value for comparison of concordant versus not concordant using t test or chi square test as appropriate